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Deciphering the morphological variability in grape germplasm

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ABSTRACT

The aim of present study was to evaluate grape accessions that have been under cultivation in India for a long time. A high variability was found in the evaluated grape germplasm for the measured parameters. Mature leaf, shoot tip, bunch parameter, berry parameter, total soluble solids (TSS), and total acidity (TA) showed a wide variation. Significant positive correlation between bunch and berry traits and negative correlation between TSS and TA was recorded. According to the present finding, grape germplasm such as Aledo, Beni Zuiho, Crimson Seedless, Banqui Abyad, Kishmish Chernyi, Beauty Seedless, Blush Seedless, Arka Soma, Black Damas Rose and Convent Large Black showed superior fruit traits. Cluster analysis and principal component analysis (PCA) showed a considerable fruit diversity in the studied grape germplasm. The characteristics of grape bunches and berries play a crucial role in quality evaluation, particularly for table grapes. The obtained data revealed phenotypic and genotypic variation within grape germplasm might be considered as characterizing gene pool that can contribute to the future grape breeding program.

Key words: *Vitis vinifera* L., ampelographic characters, diversity, principal component analysis, breeding.

INTRODUCTION

Grape (*Vitis vinifera* L.), the most valuable fruit crop in the world belongs to the family Vitaceae, containing about 60 interfertile wild species (Somkuwar *et al.,* 15). Two subspecies are available *i.e.*, *V. vinifera* L. subsp. *sylvestris* (Gmelin) Hegi and *V. vinifera* L. subsp. *sativa* (DC.) (Abiri *et al.,* 1). A great majority of *V. vinifera* L. subsp. *vinifera* cultivars widely cultivated for fruit, juice, and wine, derived from wild forms *V. vinifera* L. subsp. sylvestris (Khadivi-Khub *et al.*, 6). Genetic diversity is a valuable resource for the conservation of plant genetic resources for current and future use in breeding programs. Diversity evaluation is a crucial component of germplasm characterization and conservation, which in turn is essential for the maintenance and improvement of agricultural production, sustainable development, and poverty alleviation. Genetic diversity in crop species can be determined by using morphological, agronomical, biochemical characterization and DNA markers. It is well-known that genetic variation is needed for crop improvement and understanding gene function, which also applies to grapevine. Rohlf *et al.* (11) reported that morphological differences exist among plant species. Fruit traits have been the primary traits used to characterize fruit plants. Morphological traits combined with multivariate statistical methods such as PCA and cluster analysis have been used to assess variation and relationships among grape cultivars (Abiri *et al.,* 1; Vafaee *et al.,* 17).

India is one of the most suitable places in the world for the diversity and cultivation of grapes. However, the wide diversity in the cultivated grapevine remains unexplored. To conserve the current genetic pool and to use it for future breeding programs, there is a need to evaluate grape genotype for quality attributes and yield potential comprising its nutritive values. Therefore, the aim of the present study was to assess the variability of 49 grape germplasm conserved in the field gene bank of ICAR- National Research Centre for Grapes Pune.

MATERIALS AND METHODS

The present study on ampelographic and bunch characterizations of 49 grape cultivars grafted on Dogridge rootstock planted in National Active Germplasm Site at ICAR-NRC for Grapes, Pune (latitude 18°32N and longitude 73°51E) was undertaken during 2022-23. Pune has sub-tropical climatic conditions with a temperature range of 7.2°C (minimum) and 40.0°C (maximum) during the trial period. The vines were spaced at 3 m between the rows and 1.5 m between the vines and trained to a Y trellis system with single cordons trained in the horizontal direction while shoots were placed in a vertical position. The vines were maintained by following standard recommended cultural practices. Forty-five ampelographic and bunch parameters with the support of grape descriptors of International Plant Genetic Resources Institute (IPGRI *et al.,* 5) and DUS guideline (PPV&FRA, 10) were used

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to determine phenotypic variability among the available grape germplasm. Ampelographic parameters were studied after the forward pruning. Parameters related to the bunch were measured, calculated, and also visually estimated at the harvest stage (full maturity). The grape bunches were harvested after attaining TSS of 18°Brix. Quantitative parameters were measured using laboratory equipment such as a vernier caliper, weighing balance, measuring tape, and scale. Randomly fifty berries were selected from different bunches, then crushed, fruit juice was prepared and the juice was used for analysis of TSS and TA. TSS was measured using a hand refractometer and acidity using 0.1N NaOH. Qualitative characteristics were considered based on rating and coding according to the descriptor list for *Vitis* species (OIV, 9). The genetic diversity and relationships between cultivars were estimated using a standardized data set. Analysis of variance was performed using SAS (SAS Institute, 12) for all the morphological traits. The mean, standard deviation and coefficients of variation for each data set were calculated to determine variability. The Pearson correlation coefficient was used to determine the correlation between fruit characters. Multivariate analysis of variance PCA using SPSS software was used to correlate relationships between the cultivars. Cluster analysis was done to understand the patterns of variation among the cultivars using the unweighted pair-group method of arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Morphological characters including mature leaf, bunch and berry traits showed significant variation across the grape germplasm. The summary of frequency distribution for the parameters is given in the Table 1. The time of bud burst revealed that 63.26 % germplasm required medium time while 22.44 % were late. The opening of young shoot tip was closed in 8, half open in 19 and fully open in 22 germplasm. Regarding the colour of upper side of young leaf blade, a wide variation was observed and majority of germplasm showed yellow with bronze spot colour (30.61%), yellow (26. 53 %) and 22.44 % cultivars having copper colour. In the present study, 93.87 % germplasm were bearing hermaphrodite flowers. The growth habit of the vine revealed that 71. 42% germplasm were erect and 14.28 % were semi-erect and horizontal in nature. The bark peeling nature of vine showed that 95.91% germplasm were peeling and only 4.08 % were non peeling in nature. The variability with respect to width of leaf blade in different genotypes might be due to their genetic makeup and interaction with the environment. In the present study, five shape of leaf blade categories *viz.*, cordate, wedge, pentagonal, circular and kidney were represented by

Table 1. Summary of frequency qualitative traits of 40 grape germplasm.

Traits	Category	No. of cultivar (s)	Percentage (%)
Time of bud burst (DAP)	Very early	1	2.04
	Early	2	4.08
	Medium	31	63.26
	Late	11	22.44
	Very late	4	8.16
Young shoot: form of shoot tip	Closed	8	16.32
	Half open	19	38.77
	Fully open	22	44.89
Young leaf: colour of upper side of blade	Green	3	6.12
	Green with bronze spots	6	12.24
	Yellow	13	26.53
	Yellow with bronze spots	15	30.61
	Copper yellow	1	2.04
	Copper	11	22.44
Time of full bloom (DAP)	Early	3	6.12
	Medium	15	30.61
	Late	21	42.85
	Very late	10	20.40
Type of flower	Hermaphrodite	47	93.87
	Female with Upright stamens	2	4.08
	Female with descending order	1	2.04
Growth habit	Erect	35	71.42
	Semi erect	7	14.28
	Horizontal	7	14.28
Bark peeling	Peeling	47	95.91
	Non peeling	2	4.08
Leaf: width of blade	Medium	1	2.04
	Large	31	63.26
	Very large	17	34.69
Leaf: shape of blade	Cordate	7	14.28
	Wedge-shaped	13	26.53
	Pentagonal	15	30.61
	Circular	12	24.48
	Kidney shaped	2	4.08
Leaf: number of lobes	Single	1	2.04
	Three	11	22.44
	Five	30	61.22
	Seven	5	10.20
	More than seven	2	4.08

Contd...

Table 1 contd...

Traits	Category	No. of cultivar (s)	Percentage (%)
Leaf: anthocyanin coloration of main vein on lower side of blade	Absent	16	32.65
	Point	14	28.15
	1st bifergation	13	26.53
	2nd bifergation	6	12.24
Leaf: shape of teeth	Both sides straight	25	51.02
	Both sides convex	23	46.93
	Mixture of both sides straight and both sides convex	1	2.04
Leaf: erect hairs between veins on lower side of blade	Absent	40	81.63
	Low	2	4.08
	Very low	2	4.08
	Medium	3	6.12
	High	1	2.04
	Very high	1	2.04
Bunch density	Loose	7	14.28
	Medium	31	63.26
	Compact	11	22.44
Bunch: shape	Globular	6	12.24
	Cylindrical	16	32.65
	Conical	19	38.77
	Winged cylindrical	4	8.16
	Winged conical	3	6.12
	Double clustered	1	2.04
Berry shape	Oblate	1	2.04
	Round	15	30.61
	Short elliptical	20	40.81
	Long elliptical	8	16.32
	Cylindrical	1	2.04
	Ovate	2	4.08
	Obovate	2	4.08
Berry: colour of skin	Green- yellow	21	42.85
	Rose	1	2.04
	Red	11	22.44
	Purple	2	4.08
	Blue-blackw	14	28.56
Berry: flavour	Neutral	21	42.85
	Muscat	14	28.57
	Foxy	9	18.36
	Other	5	10.20

7,13,15,12 and 2 germplasm respectively. Five lobes were observed in the mature leaves of the majority of cultivars and agreed with the finding of Vafaee et al., (17). Out of 49 germplasm, anthocyanin coloration of main vein on lower side of blade was absent in 16, present at point in 14, up to 1st bifergation in 13 and up to 2nd bifergation in 6 germplasm. The present results are in agreement with the finding of Abiri *et al*. (1). Teeth shape of mature leaves was predominantly both sides straight (51.02%). Vafaee *et al*. (17) reported that teeth shape of mature leaves was predominantly mixture of straight and convex on both sides. Density of erect hairs between veins was absent, very low, low, medium, high and very high in which absent was predominant (81.63). The cultivars were clustered into three group based on bunch density including loose (7), medium (31) and compact (11). Bunch density is important for table grapes, as very dense bunches are often damaged during packing and transport. Seven types of berry shape were observed including oblate (1), short-elliptical (20), long elliptical (8), round (15), cylindrical (1), ovate (2), and obovate (1). Khadivi-Khub *et al*. (6) observed three types of berry shape including oblong, elliptic, and round. In addition, 5 types of berry skin color were observed and green-yellow (21) colors were predominant. Khadivi-Khub *et al*. (6) and Vafaee *et al*. (17) identified nine and four color categories for Iranian grapes. Berry skin color is an important indicator of fruit ripeness and prediction of harvest date of some fruits. Furthermore, cultivars with different berry skin color can be satisfying for various consumer preferences (Vafaee *et al*., 17). Four distinct berry flavor such as neutral (21), muscat (14), foxy (9) and other (5) were reported.

The magnitude of variability present in various quantitative traits under study revealed existence of wide range of variability among all the traits (Table 2). In the present study, grape germplasm such as Aledo, Beni Zuiho, Crimson Seedless, Banqui Abyad, Kishmish Chernyi, Beauty Seedless, Blush Seedless, Arka Soma, Black Damas Rose and Convent large Black showed superior fruit traits. Maximum bunch weight was recorded in Kishmish Chernyi (552.5g), Beni Zuiho (536.4g), Crimson Seedless (529.5g) and Banqui Abyad (521.6g) while minimum was recorded in Hussain Black Kabuli (78.5g), Jahfari (83.7g), Malaga (85.6g) and Black Muscat (98.6g). Highest 50-berry weight was observed in Katta Kurgan (453.0g) followed by Aledo (417.0g) while lowest in Barbarossa (58.0). The highest berry diameter was recorded in Aledo (23.3mm), Banqui Abyad (22.3mm), Italia (20.4mm) while lowest was noted in Barbarossa (11.6mm), Tigrosa (13.7mm). The highest TSS was noted in Crimson Seedless (21.6) followed by Beni Zuiho (21.4). The results of the present study were in

Table 2. Fruit quality attributes of different grape accessions.

Cultivar	BW	BL	BuW	BeW	BD	BeL	TSS	TD
Aledo	398.4	148.0	138.0	417.3	23.3	23.4	16.8	0.53
Arka Chitra	361.7	183.0	99.7	265.7	16.6	18.3	18.3	0.65
Golden Queen	233.7	148.0	93.3	172.0	16.3	16.7	16.4	0.53
Katta Kurgan	380.3	107.0	109.0	453.0	23.3	14.1	16.3	0.62
Katta	313.0	158.0	114.0	208.0	16.7	15.3	18.3	0.45
Gold	361.6	190.0	130.9	217.3	16.4	19.6	19.2	0.66
Muscat of Alexandria	282.5	160.0	96.0	216.0	16.2	17.5	16.3	0.65
Beni Zuiho	536.4	130.0	100.0	316.0	17.3	19.4	21.3	0.49
Black Muscat	98.6	101.0	68.0	190.3	17.6	20.2	17.3	0.65
Cardinal	250.0	155.0	86.0	235.0	15.3	22.3	16.2	0.44
Clone 2A	185.3	95.0	83.0	75.0	13.2	15.0	18.6	0.85
Gulabi	249.6	161.0	75.0	127.0	16.3	17.2	18.3	0.57
Crimson Seedless	529.5	148.0	90.0	128.0	14.7	19.4	21.6	0.50
Italian Eliquena	289.3	159.0	68.0	303.0	18.2	21.4	19.5	0.54
Kali Sahebi	329.0	114.0	75.0	275.0	16.4	19.2	18.3	0.75
Malaga	85.6	90.0	40.0	180.0	17.6	13.4	19.3	0.67
Manik Chaman	158.5	177.0	117.0	59.0	12.6	18.3	17.5	0.65
Banqui Abyad	521.6	221.0	73.0	305.7	22.3	25.7	17.6	0.54
Goethe	213.5	92.0	22.7	148.0	17.6	19.5	19.5	0.55
Kishmish Chernyi	552.5	147.0	98.0	117.0	15.4	17.5	18.6	0.55
Kishmish Maldovsskii	340.0	152.0	108.0	144.0	16.5	20.7	18.6	0.53
Kishmish Luchistvi	368.5	156.0	90.0	169.0	14.4	17.5	19.8	0.64
Kishmish Red	357.6	148.0	90.0	113.0	13.5	17.9	20.6	0.55
Kishmish Belyi	547.2	139.0	91.0	76.4	14.3	15.7	19.5	0.43
Pearl of Csaba	495.8	187.0	68.0	106.5	15.9	19.5	20.9	0.44
Beauty Seedless	410.5	153.0	89.0	61.0	11.6	12.0	21.2	0.48
Blush Seedless	532.7	164.0	83.0	74.0	15.5	12.4	19.3	0.52
Jahfari	83.7	69.0	47.0	99.0	16.7	19.8	18.9	0.80
Hussain Black Kabuli	78.5	139.0	41.0	191.7	13.9	15.0	16.8	0.92
Athens	535.5	113.0	81.0	138.0	16.6	18.7	19.5	0.66
Arka Soma	465.5	133.0	41.0	185.0	18.5	21.5	20.3	0.67
Black Damas Rose	494.5	105.0	85.0	175.0	16.6	18.5	19.7	0.58
Barbarossa	160.5	92.0	52.0	58.0	11.6	13.4	20.6	0.65
Victoire	208.9	168.0	51.0	58.8	17.8	17.9	18.0	0.79
Tigrosa	330.5	81.0	61.0	75.0	13.7	14.5	18.5	0.64
Queen of Vineyard	340.8	117.0	54.0	109.0	14.6	15.5	20.4	0.64
Pierce	300.4	102.0	37.0	154.0	16.5	19.5	20.5	0.49
Omania Black	275.3	109.0	72.0	92.5	16.4	17.2	19.4	0.56
Motia	325.3	134.0	53.0	120.3	14.5	17.4	17.7	0.53
Convent Large Black	460.7	125.0	85.0	164.4	19.4	19.8	20.5	0.58
Coarna Alba × Thompson Seedless	251.3	107.0	42.0	132.4	16.0	18.6	20.8	0.76

Contd...

Table 2 contd...

Cultivar	BW	BL	BuW	BeW	BD	BeL	TSS	TD
Arka Hans	401.5	136.0	35.0	126.4	16.9	17.5	19.1	0.50
Leh 12	502.5	214.0	114.0	182.5	16.5	14.5	18.7	0.85
Country Bangalore	291.5	96.0	83.0	158.0	15.5	17.5	20.6	0.75
Khalili	58.7	83.0	35.0	210.4	14.6	17.6	18.7	0.79
Julsky Muscat	228.0	163.0	88.0	120.0	19.3	16.8	17.4	0.62
B'lore Blue × Chardonnay	142.9	133.0	69.0	170.0	16.6	23.5	19.4	0.75
E-7-22	373.9	141.0	118.0	323.0	17.6	22.5	17.5	0.58
Italia	254.0	153.0	79.0	172.0	20.4	18.6	16.5	0.79

BW, Bunch weight (g); BL, Bunch length (mm) ; BuW, Bunch width (mm); BeW, 50 berry weight (g); BD, Berry diameter (mm); BeL, Berry length (mm); TSS (°Brix), TD, Titratable acidity (%)

accordance with the findings of Somkuwar *et al*. (16). The characteristics of grape bunches and berries play a crucial role in quality evaluation, particularly for table grapes. Variability related to bunch and berry was determined in the studied germplasm is important for breeders which helps them to improve crop productivity.

To study the relationship between fruit traits among germplasm, a correlation was carried out (Table 3). Berry weight had a positive correlation with berry diameter (r=0.566) and berry length (r=0.586) while bunch length was positively correlated with average bunch weight (r=0.314), berry weight (r=0.158), and berry length (r=0.239). Bunch width was also positively correlated with berry length (r=0.539), and berry weight (r=0.256). A significant positive correlation was observed between berry diameter and berry length (r=0.553). The result of the present investigation supports the finding of Khandavi Khub *et al.* (6). TSS showed a negative correlation with acidity (r= -0.037) and bunch width (r=-0.08). Leao *et al*. (8) also reported a negative correlation between TSS and acidity. With the increases in berry diameter, bunch weight (bunch width) also increases but reduces the total soluble solids. Increases in number of bunches per vine also reduces the TSS in grape berries (Somkuwar *et al.*, 14).

To estimate the variation between different parameters among the accession, PCA was performed. The purpose of the estimation of the correlation matrix was to reduce the data and bring consistency among the studied parameters. For each factor, a PC loading of more than 0.50 was considered as being significant, indicating six components and explaining 61.65 % of the total variance (Table 4 and Fig. 1). The first three PCs explained 46.43 % of the variance (21.19, 14.93 and 10.28%, respectively), indicating that these attributes have the highest variation between the cultivars and had the greatest impact on the distinction between them (Khadivi-Khub *et al.,* 6). The highest loading on the PC1 axis was correlated to berry diameter and titratable acidity. The parameters strongly correlated to PC2 axis were bunch length, bunch width, berry diameter and berry length while the remaining parameters showed less variability. The variables that exhibited a strong correlation with PC1 and PC2 can be regarded as indicative of bunch and berry parameters. This type of analysis essentially simplifies data sets with

Table 3. Pearson's correlation matrix between different fruit variables in studied grape germplasm.

Traits	BW	BeW	BD	BeL	TSS	TD	BL	BuW
BW	1.000							
BeW	-0.168	1.000						
BD	0.064	0.566	1.000					
BeL	0.090	0.586	0.553	1.000				
TSS	0.191	-0.309	-0.324	-0.245	1.000			
TD	-0.444	-0.048	-0.111	-0.259	-0.037	1.000		
BL	0.314	0.158	0.157	0.239	-0.226	-0.257	1.000	
BuW	0.156	0.256	0.149	0.155	-0.081	-0.203	0.539	1.000

BW, Bunch weight (g); BL, Bunch length (mm) ; BuW, Bunch width (mm); BeW, 50 berry weight (g); BD, Berry diameter (mm); BeL, Berry length (mm); TSS (°Brix), TD, Titratable acidity (%)

Table 4. Eigen values of principal component axes from the PCA of ampelographic and fruit parameters evaluated in the studied grape accessions.

Traits	Component			
	PC1	PC2	PC3	PC4
BW	-0.344	0.184	-0.445	0.017
BL	-0.343	0.533**	0.167	0.027
BuW	-0.259	0.506**	0.183	-0.124
BeW	0.245	0.473	0.078	-0.135
BD	0.512**	0.531**	-0.011	-0.056
BeL	0.255	0.567**	-0.344	0.114
TSS	-0.146	-0.309	-0.393	-0.265
TD	0.558**	-0.161	0.291	0.495
Total	15.061	10.614	7.26	5.688
% of variance	21.197	14.938	10.218	8.006
Cumulative %	36.135	46.353	54.359	61.653

** Eigenvalues are significant ≥ 0.50

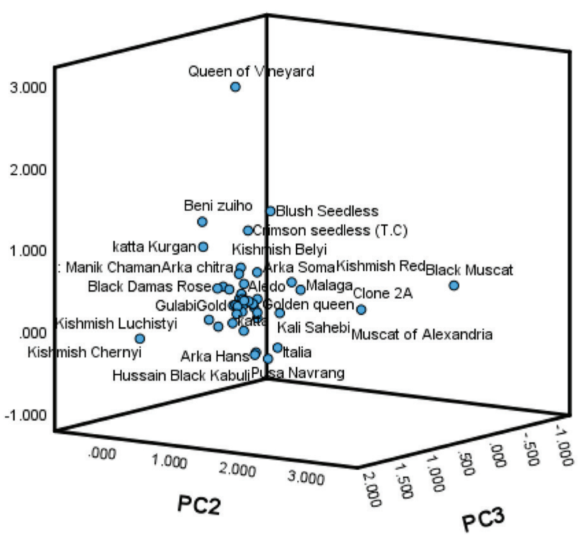

Fig. 1. Tri-plot for the studied grape cultivars based on the PC1/PC2/PC3

numerous correlated variables by transforming them into smaller sets of components. The current finding in some cases is also related to previous results in grape (Leao *et al.,* 8; Khadivi-Khub *et al.,* 6; Vafaee *et al.,* 17; Abiri *et al.,* 1). The fruit traits are the major factors that determine crop productivity and are required for crop improvements. Leao *et al.,* (8) and Khadivi-Khub *et al.* (6) also suggested bunch and berry traits as an important parameter to differentiate breeding materials linked with ampelographic parameters in grape.

Based on the morphological data, cluster analysis was carried out using an unweighted pair group method with arithmetic mean (UPGMA) to assess the similarity or dissimilarity among the different grape accessions (Fig. 2). The obtained data revealed the morphological diversity within the studied collection of grapevine accessions. In the present study, dissimilarity levels or distances ranged from 0 to 400. The dendrogram revealed two main clusters, the cluster (I) contained 15 accessions while the cluster (II) contained 34 accessions. High dissimilarity levels between the studied accessions showed high leaf and fruit variability in germplasm. The reasons for such a high dissimilarity can be explained by a low probability of gene flow between the accessions (Sefc *et al.* 13). Ekhvaia and Akhalkatsi (3) and Leao *et al.* (8) studied grape cultivars based on morphological variables and reported moderate to high variability among accessions. Villano *et al.,* (18) also reported heterogeneity in morphological traits which are under strong genetic control. Frioni *et al.* (4) demonstrated that grapevine intra-specific biodiversity hides prominent potentialities for viticulture adaptation strategies to climate change and renews the emphasis on the value of genetic resources conservation.

In the present study, 49 grape germplasm classified into different groups provides sufficient data which can be used for cultivar identification and breeding. Our results indicated that grape germplasm can be discriminated on the basis of various quantitative and qualitative traits through cluster analysis and PCA. The characterization of grape germplasm will be useful for multiplication of desirable cultivars with useful traits. This information can also be efficiently utilized in future grape improvement program for evolving superior varieties. These germplasm characterized as per the DUS descriptor will play an important role as reference cultivars for DUS testing.

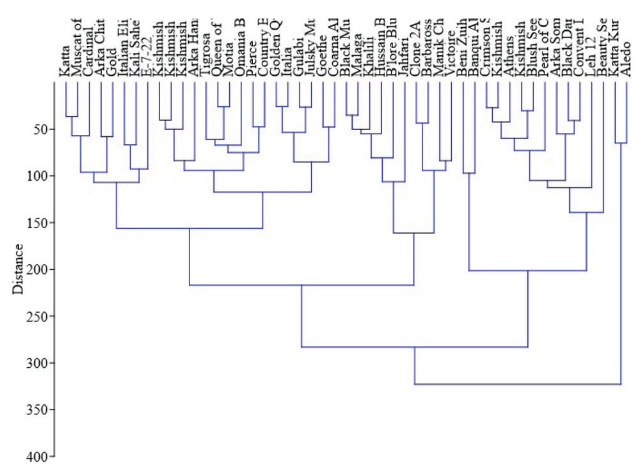

Fig. 2. UPGMA cluster analysis of the studied grape cultivars based on morphological traits using Euclidean distance.

AUTHORS' CONTRIBUTION

Conceptualization and designing of experiment (RGS, AKS, PHN and SN); field/lab experiments and data collection (RN and PK); data interpretation and preparation of manuscript (RGS, RN and AKS), statistical analysis (AAB).

DECLARATION

The authors declare that they do not have any conflict of interest.

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